

Toxicity & Teratogenicity Studies in Avian Embryos-FDA Contract #72-345

Tartaric Acid

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TARTARIC ACID

TOXICITY and TERATOGENICITY STUDIES
in Avian Embryos

FDA Contract #72-345

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STUDIES on the TOXICITY and TERATOGENICITY
of TARTARIC ACID

SUMMARY and CONCLUSIONS

Dose levels of 8 mg/kg and above were toxic to 96 hr chicken embryos when administered within the air cell or yolk under the conditions of these studies. LD-50 estimates from the mortality data indicated values of 28.5 mg/kg for the air cell-96 hr protocol, while a value of 133.5 mg/kg was obtained for the yolk-96 hr series. Statistical evaluations of the occurrence of abnormalities resulting from tartaric acid administration failed to indicate that this substance was teratogenic.

GENERAL PROCEDURES

The protocols as specified under FDA Contract #72-345 were followed in the investigation of toxicity and potential teratogenicity of the specified substance. The toxicity of the substance was evaluated from the percentage hatch of embryos injected either in the air cell or yolk at either zero hours (~~post~~^{egg}-incubation) or after 96 hours incubation to provide four separate evaluations.

EGG SOURCE AND HANDLING

All eggs used in these investigations were from Shaver Starcross pullets housed at the Poultry Research Center of the University of Arizona in Tucson. The parent stock was maintained on the University of Arizona breeder diet which had been formulated to provide more than adequate amounts of all the known nutrients required by the breeding hen.

The feed was specially prepared to assure no contaminations and did not contain any additive drugs such as antibiotics. All eggs prior to use (within 48 hours of lay) were candled to remove any containing blood spots, abnormal air cells or abnormal shells, and only clean eggs, ranging in weight from 23 - 26 ounces per dozen were used.

The supply flock was tested to assure the absence of Pullorum and Mycoplasma gallisepticum.

The eggs were incubated in forced draft Jamesway 252 machines with automatic temperature and humidity controls and an automatic turning device.

COMPOUND HANDLING FOR INJECTION

The substance tested was solubilized in a number of the prescribed solvents in order to determine the maximum concentrations which could be employed. Where possible, water was the solvent of choice. Maximum

injection volume was 0.05 ml. and all solvents and glassware were autoclaved prior to preparation of the solutions for use. The dose levels were administered with a microliter syringe using sterilized needles.

The preliminary range-finding studies using each of the administration routes and times were carried out with 10 - 25 eggs per dose level and included solvent controls, untreated controls and either drilled or pierced controls.

The actual dose-response protocol was carried out in two or more injections on different days to produce a minimum of 100 eggs at each dose level in five or more levels selected from the range-finding studies.

EXAMINATIONS OF EMBRYOS AND CHICKS

Eggs were candled daily and the dead embryos removed, examined and any abnormalities recorded. Five chicks from each dose level in each hatch were X-rayed to determine any skeletal abnormalities. Additional eggs injected at the approximate LD-50 level and an additional level below that were incubated and embryos at 8, 14, 17 days and hatch chicks removed for histopathological examinations.

In additional studies representative chicks from the dose-response protocol were saved. These chicks were housed in electrically-heated battery brooders with raised wire floors and fed University of Arizona diets. Feed consumption and growth rates were evaluated at 6 weeks of age and a sample of the birds sacrificed for gross and histopathological examinations.

DATA HANDLING

All data were coded on forms provided by FDA for computer input. In addition to summaries of mortalities and abnormalities, a number of statistical evaluations were carried out. These statistical analyses included the following for both mortality and the incidence of abnormal embryos:

1. Chi-square tests for all dose levels and for each level against the solvent control.
2. Linear regression analyses + chi square test of linearity.
 - a. % response against dose
 - b. % response against log dose
 - c. log % response against dose
 - d. arcsin transformation against dose
 - e. arcsin transformation against log dose
3. Log dose against Probit using Finney's maximum likelihood method.
 - a. Where significant, the LD-30, 50, 70 and 90's were estimated with 95% confidence intervals.
4. One-way analyses of variance.
5. Linear regression with replication.

Tartaric Acid (71-55) was solublized in water to produce a maximum concentration of 200 mg/ml for use in the four test protocols. A maximum dose of 200 mg/kg (10 mg/egg) was used.

MORTALITY

Mortality data for the four test protocols are shown in Tables 1 - 4. Maximum mortality of 86.22% was obtained with the 200 mg/kg dose level upon air cell administration at 96 hrs. Tartaric acid was found to be toxic to 96 hr embryos when injections were carried out prior to incubation (Table 5). Chi-square analyses of mortality data indicate that 8 mg/kg produced significant increases in embryo mortality employing the 96 hour injection time with either air cell or yolk administration routes. Only the highest dose level (200 mg/kg) increased embryo mortality when injected prior to incubation in the air cell. Tartaric acid was apparently well tolerated by the yolk administration route at 0 hrs (Table 5).

LD-50 estimates for the 96 hr injection times, using either air cell or yolk administration suggest that the air cell route was more toxic than when yolk administration was employed (Table 6). These results obtained from the probit analyses program indicate an LD-50 value for air cell-96 hrs of 28.5 mg/kg, while the value for yolk administration - 96 hr, was 133.5 mg/kg (Table 6).

TERATOLOGY

The data on occurrence of abnormal embryos and those showing H-S-V-L abnormalities are enumerated in Tables 1 - 4.

Statistical evaluations of these data employing chi-square analyses failed to demonstrate a significant increase in the incidence of

abnormalities in association with any of the dose levels employed in any of the four test protocols (Tables 7 & 9). Probit analyses of these data also indicated a non-significant linear relationship between log dose and probit of abnormality incidence (Table 8). Individual findings are reported in Table 10.

The results of these studies indicate that tartaric acid was not teratogenic in chicken embryos at the dose levels employed in these studies.

TABLE I
TARTARIC ACID
In WATER
AIR CELL - 0 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal		Abnormalities by category															
				Total		H-S-V-L		Head	Skeletal	Viscera	Limbs	Struc- tural	Toxic Response	Functional							
				%	#	%	#								%	#	%	#	%	#	%
200.0	139	37.41	52	2.15	3	2.15	3	0.71	1			0.71	1	0.71	1			0.71	1		
80.0	101	22.77	23	2.97	3	3.96	4	1.98	2			1.98	2								
40.0	79	18.98	15	2.53	2	0.00	0									1.26	1	1.26	1		
8.0	100	13.00	13	1.00	1	0.00	0											1.00	1		
4.0	126	19.84	25	3.96	5	3.17	4	3.17	4							0.79	1				
0.0	142	12.67	18	2.11	3	0.00	0									0.70	1	1.40	2		
drilled	40	22.50	9	0.00	0	0.00	0														
untreated	323	12.69	41	0.92	3	0.30	1	0.30	1							0.30	1	0.30	1		

SUMMARY - ALL DOSE LEVELS

545	23.49	128	2.57	14	2.02	11	1.28	7		0.55	3	0.18	1	0.37	2	0.55	3		
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TABLE 2
TARTARIC ACID
In WATER
AIR CELL - 96 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal		Abnormalities by category							
				Total	H-S-V-L	Head % #	Skeletal % #	Viscera % #	Limbs % #	Struc- tural % #	Toxic Response % #	Functional % #	
				% #	% #								
200.0	167	86.22	144	0.59 1	0.59 1	0.59 1							
80.0	169	68.63	116	1.77 3	1.77 3	0.59 1				1.18 2			
40.0	196	64.79	127	1.53 3	1.53 3	1.02 2			0.51 1				
8.0	164	39.02	64	1.82 3	0.00 0						1.21 2	0.60 1	
4.0	129	22.48	29	3.10 4	1.55 2				0.77 1	0.77 1	0.77 1		1.55 2
0.0	162	15.43	25	0.61 1	0.61 1				0.61 1				
drilled	98	14.28	14	2.04 2	0.00 0						2.04 2		
untreated	323	12.69	41	0.92 3	0.30 1	0.30 1					0.30 1	0.30 1	

SUMMARY - ALL DOSE LEVELS

825	58.18	480	1.70 14	1.09 9	0.48 4		0.24 2	0.36 3	0.36 3	0.12 1	0.24 2
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TABLE 3
TARTARIC ACID
in WATER
YOLK - 0 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal				Abnormalities by category													
				Total		H-S-V-L		Head		Skeletal		Viscera		Limbs		Struc- tural		Toxic Response		Functional	
				%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#		
200.0	188	42.55	80	1.59	3	2.12	4	1.06	2			1.06	2								
125.0	35	62.85	22	0.00	0	0.00	0														
80.0	188	50.00	94	0.53	1	0.53	1	0.53	1												
40.0	188	45.74	86	1.59	3	1.06	2					1.06	2					0.53	1		
8.0	187	47.05	88	1.06	2	0.53	1					0.53	1					0.53	1		
4.0	188	44.68	84	0.00	0	0.00	0														
0.0	221	44.79	99	0.00	0	0.00	0														
pierced	186	45.69	85	0.53	1	0.53	1						0.53	1							
untreated	323	12.69	41	0.92	3	0.30	1	0.30	1						0.30	1	0.30	1			

SUMMARY - ALL DOSE LEVELS

974	46.61	454	0.92	9	0.82	8	0.31	3		0.51	5				0.21	2
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TABLE 4
TARTARIC ACID
in WATER
YOLK - 96 HRS

Dose, ppm	No. Fertile	Mortality % #		Abnormal		Abnormalities by category												
				Total	H-S-V-L	Head	Skeletal	Viscera	Limbs	Struc- tural	Toxic Response	Functional						
				% #	% #													
200.0	88	67.04	59	1.13	1	0.00	0							1.13	1			
80.0	87	54.02	47	0.00	0	0.00	0											
40.0	88	42.04	37	2.27	2	1.13	1	1.13	1				1.13	1				
8.0	120	36.66	44	0.83	1	0.00	0									0.83	1	
4.0	130	26.92	35	4.61	6	1.53	2			1.53	2		0.76	1	0.76	1	2.30	3
0.0	165	18.78	31	1.21	2	1.21	2	0.60	1		0.60	1						
pierced	107	24.29	26	3.73	4	4.67	5	1.86	2		1.86	2	0.93	1				
untreated	323	12.69	41	0.92	3	0.30	1	0.30	1				0.30	1	0.30	1		

SUMMARY - ALL DOSE LEVELS

513	43.27	222	1.95	10	0.58	3	0.19	1		0.39	2		0.39	2	0.39	2	0.78	4
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TABLE 6
TARTARIC ACID
In WATER
PROBIT ANALYSES - MORTALITY

	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
LD-30 (Range)	NS	10.2 (0.5 - 24.6)	NS	32.5 (5.9 - 91.4)
LD-50 (Range)	NS	28.5 (6.7 - 69.6)	NS	133.5 (53.0 - 2508.6)
LD-70 (Range)	NS	70.9 (35.0 - 531.2)	NS	548.4 (156.8 - 209998.8)
LD-90 (Range)	NS	354.1 (119.3 - 32179.7)	NS	4217.6 (578.4 - ---)

TABLE 7
TARTARIC ACID
In WATER
CHI-SQUARE ANALYSES of ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
4.0	0.28	1.36	0.00	2.03
8.0	0.03	0.24	0.69	0.08
40.0	0.07	0.10	1.70	0.01
80.0	0.00	0.21	0.01	0.08
125.0	-	-	0.00	-
200.0	0.15	0.47	1.70	0.31
All Doses (DF)	2.30(5)	4.16(5)	7.31(6)	8.85(5)

TABLE 9
TARTARIC ACID
in WATER
CHI-SQUARE ANALYSES OF HLSV ABNORMALITIES

Dose Level mg/kg	Air Cell		Yolk	
	0 hrs	96 hrs	0 hrs	96 hrs
4.0	2.67	0.04	0.00	0.07
8.0	0.00	0.00	0.01	0.24
40.0	0.00	0.10	0.68	0.31
80.0	2.18	0.21	0.01	0.08
125.0	-	-	0.00	-
200.0	1.39	0.47	1.70	0.09
All Doses (DF)	9.43(5)	4.09(5)	6.67(6)	4.03(5)

TABLE 10

[illegible]

TABLE 10

[illegible]

TABLE 10

TARTARIC ACID in WATER
TERATOGENIC FINDINGS

TERATOGENIC FINDINGS			
TREATMENT	TOTAL NO. EXAMINED	TOTAL NO. ABNORMAL	SPECIFIC FINDINGS
			NO. D E S C R I P T I O N
Air Cell - 0 hrs 4.0 mg/kg	126	5	1 agenesis-head
			1 anophthalmia-unilateral; agenesis-eyelid, unilateral
			2 anophthalmia-unilateral; dysgnathia-beak
			1 dwarfism
0.0	142	3	1 hypopigmentation-down
			1 agenesis-down
			1 hemorrhage-umbilical cord
Air Cell - 96 hrs 200.0 mg/kg	167	1	1 dysgnathia-beak
80.0	169	3	2 abnormal curvature-toe, bilateral
			1 cyclopia(three eyes); acrania; dysgnathia-beak; malformation-two mandibles
40.0	196	3	1 celosomia-abdomen
			1 buphthalmia-unilateral; anophthalmia-unilateral; dysgnathia-beak
			1 dysgnathia-beak

TABLE 10

TARTARIC ACID in WATER TERATOGENIC FINDINGS

[illegible]

TABLE 10

TARTARIC ACID In WATER
TERATOGENIC FINDINGS

[illegible]